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A Review on the Possibilities of the Application of Bioremediation Methods in the Oil Spill Clean-Up of Ogoni Land

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ABSTRACT:
Many operations in the petroleum exploration, production and transportation have the potential to affect the environment in different degrees. Leakages from pipelines, oil wells, underground storage tanks of gas stations, improper disposal of petroleum wastes and stranded oil spills are the major sources of surface and groundwater contamination. There are physical, chemical, and biological methods of oil remediation. The biological method is also known as bioremediation. Bioremediation is a proven alternative treatment tool that can be used in certain oil-contaminated environments. During the process, microorganisms usually bacteria, algae and fungi, feed on the contaminants. They derive nutrition and energy for growth and reproduction. Specific indigenous microbial communities are likely to contain microbial populations of differing taxonomic relationships which are capable of degrading crude oil spill. The most important genera of hydrocarbon utilizers in aquatic environment include *Pseudomonas*, *Achromobacter*, *Arthrobacter*, *Micrococcus*, *Nocardia*, *Vibrio*, *Acinetobacter*, *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Candida*, *Rhodotorula*, *Sporobolomyces*, cyanobacteria and some algal species such as *Oscillatoria* sp., *Microcoleus* sp., *Anabaena* sp., *Agmenellum* sp., *Coccochloris* sp., *Nostoc* sp., *Aphanocapsa* sp., *Chlorella* sp., *Dunaliella* sp., *Chlamydomonas* sp., *Ulva* sp., *Cylindretheca* sp., *Amphora* sp., *Porphyridium* sp., and *Petalonia.* The use of molecular techniques in producing genetically modified organisms under strict non-proliferation rules provides a huge advantage in improving the use of microorganisms in oil biodegradation. This larger population then is able to degrade the toxins in the oil. This paper aimed at reviewing the possibilities of the application of bioremediation methods in the clean-up of oil spill in Ogoni land and the Niger Delta region at large, in comparison to the world major oil spills recorded with evaluation of the efficacy of the technologies employed.
Keywords: Bioremediation, Ogoni land, Oil spill, microorganisms, biodegradation

INTRODUCTION:

Ogoni is a tropical wetland in the Niger Delta covering an area approximately 1,000km^2 and occupies about 1% of the Niger Delta total land area, less than 2% of the Niger Delta population [1]. They live in close-knit rural communities, their livelihoods based on agriculture and fishing. The total population of the four local government areas (LGAs) – Eleme, Gokana, Khana and Tai – according to the 2006 National Census was approximately 832,000. The Ogoni people gained global prominence in the past two decades as a result of their agitation over acclaimed neglect and deprivation by the Federal Government of Nigeria as well as the level of environmental degradation in the region. The Ogoni people have been acclaimed to be victims of human right violation for many years. In 1956, four years before Nigerian Independence, Royal Dutch in collaboration with the British government found a commercially viable oil field on the Niger Delta and began oil production in 1958. Exploration and production of crude oil occurred in Ogoni land up till 1992. Oil Production in Ogoni was stopped abruptly since 1993 till date as a consequence of the Ogonis agitation and protest against the Federal Government of Nigeria for alleged environmental degradation and political alienation. Denial of access to facilities has been a major challenge thereafter and at shut down in 1993, facilities were exposed to frequent sabotage resulting in fires, oil theft and illegal oil refining leading to significant environmental degradation (spills) [1].

However, in July 2006, United Nations Environment Programme (UNEP) received an official request from the Federal Government of Nigeria to conduct a comprehensive assessment of the environmental and public health impacts of oil contamination in Ogoni land, Rivers State, together with options for remediation. In August 2011, the UNEP published an 'Environmental Assessment of Ogoni land' (popularly referred to as UNEP Report). The UNEP report estimated that it could take up to 30 years to rehabilitate Ogoni land to its full potential and that the first five years of rehabilitation would require funding of about US$1 billion. The analysts examined in the study included certain groups of hydrocarbons that are known to have adverse impacts and which are therefore dealt with selectively in oil-spill assessment and clean – up work. The important of these are BTEX (Benzene, Toluene, Ethylbenzene and Xylenes) and PAHs (Polycyclic Aromatic Hydrocarbons). Volatile organic compounds (Vocs) were the main target of UNEP's air quality investigations [2]. After 5years of this report, on June 2016, the Federal Government of Nigeria launched the formal implementation of the UNEP report and also in July they set up governing boards to monitor and supervise the

implementation of the clean-up and the remediation process.

Oil spill pose an immediate threat to the environment and require quick and thorough responses. The remediation of oil spills has to do with getting rid of the oil in order to avoid or reverse environmental damages. It is important to start removing oil promptly from contaminated areas because as time passes and the oil weathers, it causes more damage to the resources in its path [3].

Remediation of oil polluted environments is a difficult matter and can be very costly when using the following conventional methods of oil clean-up such as; use of dispersants, manual removal, use of chemicals, burning, cutting vegetation, passive collection sorbents, debris removal, trenching, removal of sediment, slurry and blasting and others[4]. According to reference [5] the damages from oil polluted environments are practically irreparable. The conventional methods above used to decrease the effects of oil spillage in the environment are usually a form of palliative solution and never definitive for the problems caused.

Due to the detrimental drawbacks of oil spills incidents which are becoming more rampant in developing countries scientists, researchers and environmentalists have developed new methods and forms of technology to facilitate the tasks of cleaning up oil spills [6]. One promising treatment method is to exploit the ability of microorganisms to remove these organic pollutants from contaminated sites. This is an alternative treatment strategy that is effective, minimally hazardous, economical, and versatile and environment friendly, is the process known as bioremediation [7].

Bioremediation methods are currently receiving favorable publicity as promising environmental friendly treatment technologies for the remediation of hydrocarbons. Moreover, biological methods can have an edge over the physico-chemical treatment regimes in removing spills as they offer cost effective *in situ* biodegradation of oil fractions by the microorganisms [8].

Crude oil and its various factions are used in virtually all aspects of any society in developing countries ranging from transportation, construction, generators and cosmetics to various forms of commercial activities. All this is done without a comprehensive evaluation of impacts of these activities to the environment and to the health as in the case of oil spills. Oil spills have become a thing of great concern in developing countries. Oil spills in Nigeria have been a concerning matter for the Nigerian population, especially in the Niger-Delta region. Oil spills may occur for numerous reasons such as equipment failure, disasters, deliberate acts or human error [9]. Oil spills on the land and sea has been on the increase with explorative activities in the Niger Delta region of Nigeria.

This paper aimed at reviewing the possibilities of the application of bioremediation methods in the clean-up of oil spills in Ogoni land and the Niger Delta region at large in comparison to the world major oil spills recorded and the evaluation of the efficacy of bioremediation technologies employed.

BIOREMEDIATION

Reference [10] defines bioremediation as any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. Bioremediation technology using microorganisms was reportedly invented by George M. Robinson [11]. He was the Assistant County Petroleum Engineer for Santa Maria, California. During the 1960s, he spent his spare time experimenting with dirty jars and various mixes of microbes. The process by which microorganisms achieve oil bioremediation is by first being able to utilize these organic pollutants for their own metabolism by means of biodegradation.

According to reference [12], biodegradation is the process by which organic substances are broken down into smaller compounds by the enzymes produced by living microbial organisms. The microbial organism transforms the substance through metabolic or enzymatic processes. The biogeochemical capacities of microorganisms seem almost limitless, and it is often said that microorganisms are “Earth’s greatest chemists” [13]. The activities of these great little chemists have been exploited in many ways. Furthermore, reference [14] sees biodegradation as the biologically catalyzed reduction in complexity of chemical compounds. It is based on two processes: growth and co-metabolism. The underlying principle behind microbial biodegradation of oil polluted environments is the ability to exploit the metabolic activities of these microorganisms in

their natural environments where their nutritional and physical requirements can be influenced.

Microbial biodegradation should not be confused with microbial biodeterioration, where biodegradation is a useful activity vital for the recycling of matter, biodeterioration is an unfavourable activity which leads to the spoilage of an object or material by microorganisms that is still useful [15]. Hence, biodegradation is a positive activity while biodeterioration is a negative activity. According to reference [16], biodegradation can be used to describe three major changes in a molecule, it could either describe a minor change in the functional groups attached to an organic compound, such as the substitution of a hydroxyl group for a chlorine group or it could refer to an actual breaking of the organic compound into organic fragments in such a way that the original molecule could be reconstructed and finally it can describe the complete degradation of an organic compound to minerals, otherwise known as biomineralisation.

MICROORGANISMS IN BIODEGRADATION OF CRUDE OIL SPILLS

Crude oil is a liquid mixture of a variety of hydrocarbon compounds derived from ancient algal and plant remains and found in reservoirs under the earth's surface [17]. From the hundreds of individual components, several classes, based on related structures, can be recognized. Since these hydrocarbons from crude oil are naturally occurring organic compounds, it is of little or no surprise that microorganisms have evolved the ability to utilize these compounds. A diverse group of microorganisms have been implicated in petroleum hydrocarbon biodegradation in both aquatic and terrestrial habitats [18,19].

When natural ecosystems are contaminated with petroleum hydrocarbons, the indigenous microbial communities are likely to contain microbial populations of differing taxonomic relationships which are capable of degrading the contaminating hydrocarbons [20]. According to reference [17], microbes that use oil as their source of energy have been around for hundreds of millions of years indeed, for as long as this energy rich substance has been available. Where oil is naturally present, for example, on the floor of the Gulf of Mexico, the community of microbes that collectively feeds on all the different compounds contained in the oil is well established and diverse [17]. Even where the background levels of oil are low, a few microbes with the capability of degrading oil always seem to be present. The ability to metabolize oil is displayed by many different types of microbes, some more versatile than others.

Research done by reference [21] on rate of biodegradation of crude oil by microorganisms isolated from oil sludge environment showed that individual microbial species of *Bacillus subtilis, Micrococcus varians* and *Pseudomonas aeruginosa* degraded oil at different rates at 72.3%, 85.7% and 97.2% respectively, with *P. aeruginosa* having the highest rate of degradation. Complex mixtures of petroleum hydrocarbons, such as crude oil and refinery sludge can be expected to alter the activity and structure of natural microbial communities [22]. Reference [23] found out that the proportion of hydrocarbon degrading microorganisms increases substantially upon exposure to hydrocarbons, reflecting the selectivity of carbon source by the microorganisms.

The ability to degrade petroleum hydrocarbons is not restricted to a few microbial genera; a diverse group of bacteria and fungi have been shown to have this ability [20]. Reference [24] in his review noted that more than 100 species representing 30 microbial genera had been shown to be capable of utilizing hydrocarbons. In a previous review, reference [25] listed 22 genera of bacteria, 1 algal genus, and 14 genera of fungi which had been demonstrated to contain members which utilize petroleum hydrocarbons; all of these microorganisms had been isolated from an aquatic environment. The most important (based on frequency of isolation) genera of hydrocarbon utilizers in aquatic environments were *Pseudomonas*, *Achromobacter*, *Arthrobacter*, *Micrococcus*, *Nocardia*, *Vibrio*, *Acinetobacter*, *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Candida*, *Rhodotorula* and *Sporobolomyces* [25].

Some cyanobacteria and algae have been tested and found to be capable of utilizing hydrocarbon from crude oil factions. Experiments carried out by reference [26] described a hydrocarbon utilizing achlorophyllous strain of the alga *Prototheca*. Also, reference [27] tested nine cyanobacteria, five green algae, one red alga, one brown alga, and two diatoms for their ability to oxidize naphthalene. They found that *Oscillatoria* sp., *Microcoleus* sp., *Anabaena* sp., *Agmenellum* sp., *Coccochloris* sp., *Nostoc* sp., *Aphanocapsa* sp., *Chlorella* sp., *Dunaliella* sp., *Chlamydomonas* sp., *Ulva* sp., *Cylindretheca* sp., *Amphora* sp., *Porphyridium* sp., and *Petalonia* all were capable of oxidizing naphthalene. Their

results indicate that the ability to oxidize aromatic hydrocarbons is widely distributed among the cyanobacteria and algae [20]. The table below shows predominant bacterium in soil samples polluted with aliphatic and Aromatic Hydrocarbons, Polycyclic Aromatic hydrocarbon and Chlorinated compounds.

Table 1: Representative Bacteria with Broad Hydrocarbon Biodegradative Abilities

Gram negative bacteria	**Gram positive bacteria**
Pseudomonas sp.	*Norcardia* sp.
Acinetobacter sp.	*Mycobacterium* sp.
Allcaligenes sp.	*Corynebacterium* sp.
Flavobacterium sp.	*Arthobacter* sp.
Xanthomonas sp.	*Bacillus* sp.

Source: [28]

1. The Genus *Pseudomonas*

Pseudomonas is an aerobic gram negative rod shaped bacterium, it shows no fermentative activities and seem to have the highest degradative potential e.g., *Pseudomonas putida* and *P. fluorescens* [28]. Although, a wide phylogenetic diversity of bacteria is capable of degradation of pollutants, *Pseudomonas* sp. and closely related organisms have been the most extensively studied owing to their ability to degrade so many different contaminants [29]. The immense potential of the *Pseudomonads* does not solely depend on the catabolic enzymes, but also on their capability of metabolic regulation [29]. The presence of multiple dioxygenases in *Pseudomonas* sp. and related strains dramatically expands the range of substrates capable of being catabolized [30]. The dioxgenases which act on aromatic hydrocarbons are dramatically broad in their substrate specificities [31].

2. *Rhodococcus* sp.

A second important group of degrading bacteria are the gram-positive rhodococci and coryneform bacteria. Many species, now classified as *Rhodococcus* sp. had originally been described as *Nocardia* sp., *Mycobacterium* sp., and *Corynebacterium* sp. Rhodococci are aerobic actinomycetes showing considerable morphological diversity. A certain group of these bacteria possess mycolic acids at the external surface of the cell. These compounds are unusual long-chain alcohols and fatty acids, esterified to the peptidoglycan of the cell wall. Probably, these lipophilic cell structures have significance for the affinity of rhodococci to lipophilic pollutants. In general, rhodococci have high and diverse metabolic activities and are able to synthesize biosurfactants [28].

3. *Sphingomonas yanoikuyae* B1

S. yanoikuyae B1 previously known as *Beijerinckia* sp. strain B1, was originally isolated for its ability to use biphenyl as a carbon source [31]. It was the first bacterium for which definitive metabolites were shown for multi-ring polycyclic compounds, such as benzo[a]pyrene and benzo[a]anthracene [32]. *S. yanoikuyae* B1 is remarkable as a bacterium capable of oxidizing many aromatic compounds as its source of carbon and energy [31]. As such, it is representative of the genus *Sphingomonas,* a group of gram-negative bacteria generally known for their diverse catabolic activities.

PROCESS INVOLVED IN THE USE OF MICROORGANISMS IN OIL BIODEGRADATION

Polycyclic Aromatic Hydrocarbon (PAH) compounds are usually degraded under aerobic and anaerobic conditions. In both cases, a key step is the activation of the inert aromatic ring [33]. In the presence of oxygen, this is carried out by oxygen dependent enzymes. Under anaerobic conditions, however, the rate and extent of hydrocarbon biodegradation decreases and the variety of substrates degraded is typically narrower [34, 35].

1. Aerobic Biodegradation

Reference [28] defines aerobic biodegradation as the breakdown of organic pollutants by microorganisms when oxygen is present. More specifically, it refers to a microbial catalytic process occurring or living only in the presence of oxygen; therefore, the chemistry of the system, environment or organism is characterized by oxidative conditions. Many organic contaminants are rapidly degraded under aerobic conditions. Aerobic microorganisms have an oxygen based metabolism where aerobes through cellular respiration, use oxygen to oxidize substrates in order to obtain energy. Before cellular respiration begins, glucose molecules are broken into smaller molecules in the cytoplasm. Oxygen of the cells is used in the chemical reactions that breakdown small molecules into H_2O and CO_2 in a reaction that releases energy.

Reference [28] reported that the most important classes of organic pollutants in the environment are mineral oil constituents and halogenated products of petrochemicals, therefore, the capacities of aerobic microorganism are of particular relevance for the biodegradation of such compounds as

exemplarily described with reference to the degradation of aliphatic and aromatic hydrocarbons as well as their chlorinated derivatives [28]. Examples of some microorganisms that carry out aerobic biodegradation include *Rhodococcus* sp., *Burkholderiaxenovorans, Pseudomonas sp.* [36].

2. Anaerobic Biodegradation

Many polluted environments are often anoxic, e.g., aquifers, aquatic sediments and submerged soils. In such environments, biodegradation is carried out by strict anaerobes or facultative microorganisms using alternative electron acceptors, such as nitrate (denitrifying organisms), sulfate (sulfate reducers), Fe (III) (ferric-ion reducers), CO_2 (methanogens), or other acceptors such as chlorate, Manganese, Chromate, and others [37,10,38]. Anaerobic biodegradation involve a series of processes in which microorganism's breakdown biodegradable materials in the absence of oxygen [39].

The use of electron acceptors other than oxygen is based on: The electron-acceptor availability and the competition of different respiratory types of microorganisms for electron donors. For example reduction of Fe(III) is the most frequent mechanism for oxidation of organic matter in subsurface environments. Sulfate is a major electron acceptor for the anaerobic degradation of contaminants in marine environments due to the high concentrations of sulfate in seawater [10].

In terms of energy, whereas degradation of aromatics using nitrate and Fe(III) as terminal electron acceptors is almost as efficient as that using oxygen, sulfate reducers and methanogenic conditions generate comparatively much less energy [40].

Consequently, the molar cell yields under methanogenic and sulfidogenic conditions are rather low. Microbial utilization of aromatic compounds. The different terminal electron acceptors in respiration are indicated in bold and they are aligned with the redox potential bar. The energetics (free-energy changes) of the aerobic and anaerobic degradation of a model aromatic compound, benzoate, are indicated on the right. Methanogenesis needs to be coupled to fermentation reactions.

3. Aerobic versus Anaerobic Microorganisms in Biodegradation

The presence or absence of oxygen often dictates the type of biodegragative pathways and the types and number of bacteria involved in the biodegradation of a particular compound [31]. In practical biodegradation, the choice of fostering aerobic or anaerobic condition is often a crucial one. Reference [31] reported that, more knowledge is accumulated about Aerobic and facultative Aerobic microorganism. Organisms such as *Pseudomonas* sp. or *Escherichia coli* can be grown overnight with simple equipment to yield high cell densities. In contrast, anaerobic enrichment cultures may initially show very long lag before significant biodegradation occurs. Upon repeated transfers, the lag phase often shortens continually. Still many years may be required to achieve significant high rates of biodegradation and they may never approach the rates of comparable aerobic biodegradation [31]. Aerobic processes typically yield more energy, generate a commensurately greater number of ATP equivalents, and generate more biomass per unit of compound transformed [41,42]. Thus, compounds with a significant energy yield upon oxygenative metabolic to carbon dioxide and water are often rapidly biodegraded under aerobic conditions.

CHALLENGES INVOLVED IN THE USE OF MICROORGANISMS IN OIL BIODEGRADATION

There are a number of challenges to the use of microorganisms for the biodegradation of oil. The biodegradation of oil pollution or spills in the environment is a complex process where quantitative and qualitative aspects depend on the nature and amount of the pollutant present, the ambient and the seasonal environmental condition, and the constitution of the indigenous microbial community [43,44]. Reference [8] carried out a research which aimed to explore the possibility of the use of selected bacterial cultures and a mixed bacterial consortium to degrade crude oil at various pH, temperatures, and oil concentrations, since extreme pH and temperature conditions are expected to have a negative influence on the ability of microbial populations to degrade the hydrocarbons [45].

Reference [8] found out that microorganisms show best biodegradative potential at optimum Temperature and pH. Hence, where these environmental conditions are not at optimum for the degrading species of microbes present at the oil polluted site, biodegradation is known to occur at a less optimum rate, since the fate of hydrocarbon degradation is largely determined by the local

environmental conditions, which influence the microbial growth and enzymatic activities.

The rates of uptake and mineralization of many organic compounds by a microbial population depend on the concentration of the compound [46]. Inhibition of biodegradation may occur due to high concentrations of undispersed volatile hydrocarbons. Bacteria used to remediate pollutants probably undergo environmental stress due to high concentrations of toxic contaminants, toxic solvents, extreme pH, temperature, ionic strength, etc. [47]. To determine the effect of concentration on microbial degradation, reference [8] experimented with selected isolates and mixed bacterial consortium on various concentrations of crude oil (1, 3, 6, 9, and 12%). For all the concentrations, the experiment was conducted at 35°C and pH 7. The inoculated flasks were incubated for 25 days and bacterial growth and crude oil degradation were estimated. The effects of crude oil concentrations on the growth of individual bacterial cultures and the mixed bacterial consortium, and crude oil degradation by them were tested and the results showed that the mixed bacterial consortium had 76% degradation at 1% BH crude oil, followed by 72% at 3%, 63% at 6%, 52% at 9%, and 41% at 12%. The individual cultures also showed the good degradation potential at 1% BH crude oil and decreased degradation at higher concentrations of the crude oil. Another major challenge for the persistence of some aromatic hydrocarbon compounds in the environment is their limited bioavailability [12].

Typically, petroleum hydrocarbons present are frequently attached to soil particles, making them non bioavailable to the degrading microorganisms. The hydrocarbon degrading microorganisms produce bio-surfactants of diverse chemical nature and molecular size [48]. These surface active materials increase the surface area of hydrophobic insoluble substrates and increase their bioavailability, thereby enhancing the growth of bacteria and the rate of bioremediation. A phenomenon unique to surfactants is the self assembly of molecules into dynamic clusters called micelles [48]. Reference [49] looked for an enhancement in the biodegradation rates due to the production of surface active compounds produced by bacteria. Surprisingly, there was no improvement in the biodegradation rates in their study because the solubilising effect of surfactants is attributed mainly to the formation of micelles.

RECENT APPLICATIONS OF BIOREMEDIATION TECHNIQUES AND THEIR EFFECTIVENESS

1. *Amoco Cadiz*

In the case of the *Amoco Cadiz* spill, which contaminated large stretches of the Brittany shoreline in France in March 1978, *natural* biodegradation was found to occur rapidly. While it might have been predicted that the microbial populations in that region would be adapted to petroleum hydrocarbon degradation, since they had frequently been exposed to releases from ballast water tanks, it had not been predicted that the rates of low-molecular-weight hydrocarbon degradation would be as fast or faster than chemical evaporation and dissolution. Until that spill, it had been accepted that biodegradation occurred only after a significant lag period, typically of the order of 2-4 weeks, and that chemical and physical weathering of the oil always preceded biological weathering [50]. Besides mechanical recovery, four different bioremediation products have been applied to the beaches. They only lead to limited and inconclusive results. Some changes in oil content were found in the experiments, but it remained unclear, if the removal was physically or biologically mediated [51].

2. *Exxon Valdez*

The *Exxon Valdez* oil spillage in March, 1989 created the largest spill ever with more than 2,000 km of oiled shoreline. The clean-up efforts included removing bulk oil, manual pickup of oil sith sorbent pads, shore washing with hot, warm, and cold water, mechanical tilling, removal or oiled sediments, and bioremediation [52]. Regarding the last method, both techniques, seeding with microbial cultures and environmental modification were considered as bioremediation methods.

2.1 Seeding with Microbial Cultures

In the initial effort to identify cultures that might be applied to the clean-up effort in Prince William Sound, products from 10 companies were selected for laboratory phase testing by EPA. Some products delayed biodegradation. Most *natural* biodegradation, when it occurred, started after a 3-5 day lag period and reached significant levels after 20-30 days. Of the products tested, two were selected for further field testing in Prince William Sound on shorelines impacted by the spill. In the field trials, four small plots were used to assess the effectiveness of seeding. These field trials failed to demonstrate enhanced oil biodegradation by these products. There were no significant differences

between the four plots during a 27-day trial period. It must be noted, however, that the oil was already highly degraded by the time these field trials were conducted, and that environmental variability makes it difficult to observe statistically significant differences between experimental and reference sites when relatively few samples are collected and analyzed [50].

2.2 Environmental Modification

Additionally, EPA carried out a comprehensive, large-scale project applying different fertilizers to the contaminated shorelines in Prince William Sound. Its objective was to demonstrate the enhancement of biodegradation through the addition of nitrogen and phosphorus in the form of three different types of fertilizers: Inipol™ EAP22, an oleophilic fertilizer formulation, and Customblen, a granular slow-release fertilizer. Oleophilic means literally oil loving. Inipol™ contains surfactants as well as nutrients, and is designed to stick to oil on rocky substrates, providing nutrients at the oil/air interface where microbial degradation takes place. Several monitoring programs measured the effectiveness of these fertilizers in reducing oil contamination and evaluated potential environmental impacts as, for example, nutrient enrichment in adjacent waters and toxicity to marine organisms.

The most controversial aspect of bioremediation applications in Prince William Sound centered on the 2-butoxy-ethanol component in Inipol™ and its potential toxicity to wildlife and cleanup workers. This was addressed by following worker safety guidelines during application of Inipol™, and by using wildlife deterrents during the first 24hr when toxicity is of most concern [53].

Nevertheless, Inipol™ turned out to produce very dramatic results in field tests, stimulating biodegradation so that the surfaces of the oil-blackened rocks on the shoreline turned white and appeared to be free of surface oil within 10 days after treatment [54]. The striking visual results strongly supported the idea that oil degradation in Prince William Sound was nutrient limited and that fertilizer application was a useful bioremediation strategy [50]. Because of the success, Inipol™ was approved for shoreline treatment and used as a major part of the clean-up effort. Additionally, Customblen has been applied. In approximately 2-3 weeks, oil on the surface of cobble shorelines treated with Inipol™ and Customblen was degraded so that these shorelines were visibly cleaner than non-bioremediated shorelines. Tests demonstrated that fertilizer application sustained higher numbers of oil-degrading microorganisms in oiled shorelines and that rates of biodegradation were enhanced, as evidenced by the chemical changes detected in recovered oil from treated and untreated reference sites [55].

As a result of the EPA-Exxon and joint monitoring projects, bioremediation of oil contaminated beaches was shown to be a safe clean-up technology. The addition of fertilizers caused no eutrophication, no acute toxicity to sensitive marine test species, and did not cause the release of undegraded oil residues from the beaches [55].

Another field study concentrated on the effects of fertilizer addition. It found out that biodegradation rates mainly depend on the concentration of nitrogen within the shoreline, the oil loading, and the extent to which natural biodegradation had already taken place. The more oil has already degraded, the less likely bioremediation has found to be effective. However, because of the heterogeneity of shorelines and oiling levels, an optimum amount of fertilizer would vary with the location, and the best dosage could not be predicted *a priori* [56].

3. *Mega Borg*

Bioremediation of the open water *Mega Borg* spill off the Texas coast in June 1990 consisted of applying a seed culture produced by the Alpha Corporation. This spill was also treated with dispersants and some burning of the oil occurred. The Texas General Land Office reported that the use of the Alpha culture on the *Mega Borg* spill was effective at removing significant amounts of oil. There was, however, no systematic or independent monitoring for effectiveness. In contrast, the study demonstrated the potential problems with the application of bioremediation problems at sea [51].

4. *Apex* Barges

Biotreatment with the Alpha culture was also used in a spillage from the *Apex* Barges after an accident at Galveston Bay in Texas in July 1990. Here again, the Texas General Land Office reported that the bioremediation was effective. Independent observations, however, indicated that treated oil changed in physical appearance and may have emulsified as a result of addition of the Alpha product. Chemical analyses on samples from impacted and reference sites failed to demonstrate that treatment with the Alpha product enhanced

rates of petroleum biodegradation. No significant differences in C_{18}/phytane ratios that would indicate biodegradation enhancement were detected between Alpha-treated and untreated sites. Thus, scientifically valid conclusions cannot be reached substantiating the effectiveness of seeding of open water or coastal spills. Clearly designed and extensive experiments, with appropriate controls, will be needed if the efficacy of seeding open water oil spills is ever to be resolved [50, 51].

5. Arabian Gulf War

One experiment analyzed the effectiveness of a certain bioremediation agent in degrading the oil spilled in the Arabian Gulf. The commercially available bacterial product consisted of a mixture of naturally occurring microorganisms. The degradation of the oil was observed under different concentrations of oil, added nutrients and added bacteria [57].

The results obtained in the study have demonstrated that the addition of nutrients and bacteria to oil has enhanced the biodegradation of the *n*-alkane fraction of the oil. A lesser degree of enhancement was obtained when nutrients alone were added, and microbial degradation of oil was not significant in the absence of nutrients or bacteria. The increase of oil biodegradation with the addition of nutrients alone was believed to be attributed to the enhancement of oil biodegradation by bacteria indigenous in seawater [57].

The study also found out, that bioremediation works more effectively at low oil concentrations. At higher oil concentrations, the differences were too small to preferentially recommend the use of bacteria seeding over nutrient addition only. Another study focused on the relationship between indigenous and seeded microbial cultures. The results showed that seeding with local or foreign oil-degrading bacteria did not lead to enhancement of hydrocarbon degradation and resulted in dramatic decreases in the numbers of the predominant, indigenous, oil-degrading bacteria. Whereas local microorganisms were able to establish themselves rather easily in the Gulf coast sand, the foreign bacteria (the German *Arthrobacter* strains, KCCG 351-355) either decreased or did not survive at all. Still, they contributed to hydrocarbon degradation [58]. Overall, the experiment turned out to be successful as after one year, insects and worms inhabited the sand. The fact that the whole polluted area of Kuwait - the 50 km^2 desert - did not recover satisfactorily was found to be due to the lack of water, which is essential for the indigenous microflora. The study concludes that bioremediation could best be carried out by the indigenous microorganisms if they are properly managed, that means that dry habitats have to be watered if necessary [58].

PROSPECTS FOR THE USE OF MICROORGANISMS IN OIL BIODEGRADATION

Despite the advantages to the use of microorganisms in oil bioremediation, due to the fact that microorganisms have acquired the ability to use polyaromatic hydrocarbons as carbon and energy sources. Their efficiency at removing such pollutants might not be optimal for cleaning up present-day pollution [12]. In fact, microorganisms have evolved towards ecological fitness rather than biotechnological efficiency; thus, it would take a long time for bacteria capable of cleaning up anthropogenic pollution to evolve by natural selection [12].

New methodological breakthrough in sequencing, genomics, proteomics, bioinformatics and imaging are producing vast amount of information in the field of Environmental microbiology, genetic engineering and gene manipulation to open a new era providing unprecedented *in silico* views of metabolic and regulatory networks, as well as clues to the evolution of degradation pathways and to molecular adaptation strategies to changing environmental pollution conditions using microorganisms [12].

These novel techniques are known to effectively contribute to cost reduction, minimizing chemical use and also improving cost-benefit ratios [59]. Hence, studying the physiology, biochemistry and genetics of the catabolic pathways becomes crucial to recreate and accelerate natural processes in the test tube as well as to accomplish their rational manipulation to design more efficient biocatalysts for different biotechnological applications. These include:
(i) bioremediation of polluted sites, (ii) biotransformation of toxic compounds into fine chemicals and other high added-value products (green chemistry), and (iii) development of *in situ* biomonitoring devices and biosensors to monitor pollutant bioavailability [60,61,47].
Many PCR primers that target genes related to petroleum-degrading enzymes, both in aerobic and anaerobic conditions are now available. The

utilization of these already-characterized primers may facilitate environmental screening of degrading abilities and may help to evaluate the potentials of microbial isolates [59]. More primers can be described for specific pathways or to improve the comprehensiveness of known primers using available databases. Also, the host cell can be manipulated to enhance bioavailability of the pollutant by engineering the production of biosurfactants or by promoting chemotaxis of the biodegrader to the toxic compound [62, 63]. Surfactant production has been combined with the ability to selectively cleave carbon-sulfur bonds in the sulfur containing compounds present in oil (biodesulfurization) and, hence, efficient recombinant biodesulfurizers have been obtained [64]. Some anaerobes release Fe(III) chelators, which solubilize Fe(III) from Fe(III) oxides, and electron shuttling compounds, which accept electrons from the cell surface and then reduce Fe(III)oxides. It has been shown that enhancing the availability of some electron acceptors, such as the insoluble Fe(III)oxides, by adding suitable ligands can greatly stimulate anaerobic degradation of contaminants in subsurface environments [10].

The benefits provided by molecular tools can open unlimited windows of opportunity, as it is possible to detect genes from cultivable or noncultivable organisms (using metagenomics) and to express these genes in cultivable organisms, using enzymes that were not yet described [59]. Biosafety is a major issue when releasing recombinant microorganisms into any open environment. In order to address this concern, several genetic circuits have been developed to allow survival of the recombinant microorganism only when present in the polluted site and during the time required for removal of the pollutant (biological containment). To avoid dispersal of the recombinant trait from the recombinant bacteria to the native microbial population, different gene-containment circuits based on a toxin and its cognate antidote have also been developed. Such active containment systems reduce significantly the potential risks that release of recombinant bacteria might cause in the ecosystem [65, 66].

CONCLUSION

In conclusion, bioremediation is a proven alternative treatment tool that can be used in certain oil-contaminated environments. During the process, microorganisms usually bacteria and fungi, feed on the contaminants. They derive nutrition and energy for growth and reproduction. It is a relatively slow process, requiring weeks to months to effect cleanup. If done properly, it can be very cost-effective, although an in-depth economic analysis has not been conducted to date. This process can be aerobic or anaerobic depending on the microorganisms and the electron acceptors available. This process may be natural (intrinsic bioremediation) or it may be enhanced by man (engineered bioremediation). The noteworthy results from field monitoring of actual bioremediation applications confirmed the theoretical information base that had already been established by previous scientific studies. Researchers had often documented that indigenous microbes usually out-compete foreign or introduced strains. The addition of nutrients in the form of fertilizer to indigenous microorganisms has proved to be effective in enhancing biodegradation and environmentally safe at the same time.

It has also been observed, that microbes with the capacity to degrade oil are present in nearly all coastal environments, and that environmental parameters besides nutrients will affect actual degradation rates in the field. Thus field applications of nutrients are still to some degree influenced by temperature, water runoff, substrate, and other environmental parameters that are neither fully understood nor easily quantified. However, there still remains a role for bioremediation in marine oil spill cleanup since experience has shown that no single technique will ever be appropriate for all incidents requiring response after oil spills.

Finally, there are many advantages to be gained from a quick cleanup of an oil spill, some of which relate not to the marine ecosystem, but to other concerns. These include economic impacts from lost use of shorelines for recreation, legal liabilities and settlement of claims, and aestethic considerations. Bioremediation has proven successful on petroleum and hydrocarbon contamination. Its advantages generally outweigh the disadvantages, which is evident by the number of sites that choose to use this technology and its increasing popularity.

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